REMARKS

The Office action dated January 31, 2011 is acknowledged. Claims 1, 2, 4-14 and 16-18 are pending in the instant application. Claims 1, 2, 4, 5 and 16 have been rejected and claims 6-14, 17 and 18 have been withdrawn. By the present Office Action response, new claims 19, 20 and 21 have been added to further specify the presently claimed invention. Support for the new claims may be found throughout the specification, such as at paragraphs [0031] for claim 19, [0032] for claim 20 and [0031] for claim 21, i.e., where paragraph [0031] recites that the number of biotin binding sites per avidin molecule was 2.4 (of the 4 biotin binding sites present on each avidin molecule). It is submitted that this average number of free (functional) binding sites was determined by a titration experiment with biotin-4-fluorescein and that the resulting avidin-coupled nanoparticles were then loaded with biotinylated antibodies (last sentence of paragraph [0031] of the specification). Reconsideration is respectfully requested in light of the amendments and arguments made herein. No new matter has been added.

Rejection of Claims 1, 2, 4, 5 and 16 Under 35 U.S.C. 103(a)

Claims 1, 2, 4, 5 and 16 have been rejected under 35 U.S.C. 103(a) as being unpatentable over WO0289776 (Kreuter, et al.) in view of EP049607 (Paganelli, et al.) and Langer, et al. (*European Journal of Pharmaceutics and Biopharmaceutics*). The Examiner argues in the Office action that Kreuter, et al. teach nanoparticles comprising proteins, e.g., gelatine and human serum albumin coupled with antibodies. The Examiner also argues that Kreuter, et al. teach nanoparticles having covalently coupled avidin via which biotinylated apolipoprotein E can be bound. In turn, the Examiner argues that by the covalent linkage of the avidin to the nanoparticles, it is not only possible to bind

biotinylated ApoE, which is necessary for the transport to the blood-brain barrier, but also to bind a variety of biotinylated molecules to the avidin-modified nanoparticles in a particularly efficient manner. For this purpose, pharmacologically or biologically active molecules are especially preferred according to the Examiner.

The Examiner additionally states that Kreuter, et al. teach to impart pharmacologic effects, pharmacologically or biologically active substances are incorporated in the nanoparticles, or they are bound by the nanoparticles, where the binding of the active agents may be performed covalently with complex-formation via the avidin-biotin system, as well as incorporatively or adsorptively.

According to the Examiner, Kreuter, et al. also teach that amino groups, carboxyl groups and hydroxyl groups located on the surface of the nanoparticles can be converted by suitable reagents to reactive thiol groups, where functional proteins are bound to the thiol group-modified nanoparticles via bifunctional spacer molecules having reactivity both to amino groups and free thiol groups. In this regard, the Examiner states the functional proteins to be coupled to the nanoparticles are selected from the group comprising avidin, avidin derivatives, apolipoproteins such as apolipoprotein E and antibodies.

The Examiner acknowledges in the Office action that Kreuter, et al. fail to explicitly embody using biotinylated monoclonal antibodies bound by a stable avidin-biotin complex.

The Examiner refers to the Paganelli, et al. reference for teaching a biotinylated monoclonal antibody, or biotinylated fragments thereof, specific to a tumor-associated antigen expressed by the tumor, wherein a protein of the avidin type binds to biotin and a

biotinylated monoclonal antibody is administered to a patient, such that avidin is and subsequently administered that specifically binds to the biotinylated monoclonal antibody.

The Examiner refers to the Langer, et al. reference for teaching the preparation of avidin-labeled protein nanoparticles as carriers for biotinylated peptide nucleic acid and preparing protein nanoparticles followed by covalent linkage of avidin, wherein free sulfhydryl groups were introduced onto the surface of protein nanoparticles. The Examiner also states that the number of primary amino groups and sulfhydryl groups on the surface of the resulting particles was quantified with site-specific reagents, that avidin was attached to the surface of the thiolated nanoparticles via a bifunctional spacer and that biotinylated peptide nucleic acid (PNA) was effectively coupled to the nanoparticles by complex formation with the covalently attached avidin.

The Examiner's conclusion as set forth in the Office action is that it would have been obvious to one skilled in the art to modify the nanoparticles as taught by Kreuter, et al. to include biotinylated monoclonal antibodies that are complexed with avidin since the Kreuter, et al. reference teaches that a variety of biotinylated molecules can be complexed to the avidin-modified nanoparticles in an efficient manner. The Examiner also concludes that Paganelli, et al. explicitly teach that a biotinylated monoclonal antibody can be complexed to a protein of the avidin type and that one would have been motivated to provide a biotinylated monoclonal antibody, since it can be used to specifically target a tumor-associated antigen expressed by a tumor. Additionally, the Examiner concludes that one skilled in the art would have recognized that other biotinylated molecules, such as peptide nucleic acids, could also be complexed to

nanoparticles via an avidin complex as taught by Langer, et al. Thus, according to the Examiner, one skilled in the art would have recognized that a biotinylated monoclonal antibody could also be complexed to the nanoparticles as taught by Kreuter, et al. for the purpose of targeting the nanoparticles to a tumor site. Lastly, the Examiner concludes that one skilled in the art would have had a reasonable expectation of success to provide nanoparticles in accordance with the presently claimed invention by following the teachings of Kreuter, et al., Paganelli, et al. and Langer, et al.

The Applicants respectfully submit that to establish a *prima facie* case of obviousness, three basic criteria must be met, as set forth in M.P.E.P. § 2142. First, there must be some suggestion or motivation to modify the reference or to combine the reference teachings. Second, there must be a reasonable expectation of success. Third, the prior art reference (or references when combined) must teach or suggest all the claim limitations. The Applicants respectfully disagree with the Examiner's conclusions set forth in the present Office action. Specifically, it would not have been obvious for one of ordinary skill in the art to modify or otherwise combine the cited prior art as stated by the Examiner. Even if such combination or modification were done, each and every limitation of the presently claimed invention would not be taught or disclosed.

The prior Final Office action had rejected the claims as being anticipated by Kreuter, et al. The numerous deficiencies of Kreuter, et al., as discussed in the Final Office action response filed on September 2, 2010 are incorporated herein in their entirety. Specifically, Kreuter, et al. fail to teach nanoparticles having biotinylated antibodies bound to avidin moieties which are coupled to the nanoparticle surface via bifunctional spacer molecules. Kreuter, et al. do not teach using biotinylated antibodies.

It is respectfully submitted that Kreuter, et al. in view of Paganelli, et al. and Langer, et al. would not be obvious nor would such combination teach all the limitations of the presently claimed invention. Paganelli, et al. and Langer, et al. fail to make up for any of the numerous deficiencies of Kreuter, et al. According to the Examiner, Paganelli, et al. teach the binding of biotinylated antibody by a stable-biotin complex which is not explicitly taught by Kreuter, et al.

It has been established that an obvious to try rationale may be proper when the possible options for solving a problem were known and finite. However, if the possible options were not either known or finite, then an obvious to try rationale cannot be used to support a conclusion of obviousness. (*Rolls-Royce, PLC v. United Technologies Corp.*, 603 F.3d 1325 (Fed. Cir. 2010)). The Applicants respectfully disagree with the Examiner's conclusion and submit that the teaching of the complex by Paganelli, et al. is merely speculative. Paganelli, et al. teach certain biochemical reactions that are supposed to take place inside a human or animal body system upon sequential administration of reagents a), b) and c). Reagent a) is a biotinylated monoclonal antibody, reagent b) is a protein of the avidin type and reagent c) is a biotin conjugated with an agent, e.g., radioisotope (col. 2, lines 15-50).

It is further submitted that since the alleged reactions between biotinylated antibody and avidin take place inside the human or animal body, and since a "stable avidin complex" (as stated by the Examiner) was neither positively observed nor produced in an isolated form (*in vitro*), it is respectfully submitted that it remains purely speculative whether such complexes were ever obtained by following the *in vivo* procedure described by Paganelli, et al., and thus would not support an obvious to try

rationale.

Furthermore, since biotin is generally known to be a normal (and vital) component of the blood (see, e.g., Watanabe, et al.; "Biotin status and its Correlation with other Biochemical Parameters in the Elderly People of Japan;" Journal of the American College of Nutrition; Vol. 17, No. 1, pages 48-53 (1998) as attached), the Applicants submit that it is questionable whether "protein of avidin type" when administered in accordance with the teachings of Paganelli, et al. will form complexes with the biotinylated antibodies since the patient's serum already contains biotin. It would be expected then that the serum which already contains biotin would bind to the biotin binding sites of the "protein of avidin type" (reagent b)), thus blocking these binding sites and preventing the alleged complex formation with biotinylated antibodies. Moreover, it is submitted that the teachings of Paganelli, et al. are merely derived from theoretical considerations without providing any clear evidence.

In view of the above, the Applicants respectfully submit that the Paganelli, et al. reference cannot be relied upon for teaching "that a biotinylated monoclonal antibody can be complexed to a protein." In this regard, the Paganelli, et al. reference also does not pertain to nanoparticles that are obtainable by an *in vitro* process. Even if the method of Paganelli, et al. resulted in the formation of complex formation between biotinylated antibodies and avidin *in vivo*, this teaching could not be employed for producing antibody-coupled nanoparticles (as presently claimed) *in vitro*.

Therefore, based on the teachings of Paganelli, et al., the Applicants submit that one skilled in the art would not have had a reasonable expectation of success for arriving at the presently claimed invention as Paganelli, et al. would not have provided any

evidence supporting their proposed theoretical concept. In particular, the alleged complex formation was not positively demonstrated whatsoever by Paganelli, et al.

The Langer, et al. reference, as noted above, also fails to make up for any of the numerous deficiencies of Kreuter, et al. and Paganelli, et al. Thus, the combination of teachings of Kreuter, et al. and Paganelli, et al. along with Langer, et al. would still fail to teach each and every claimed limitation and would still fail to provide a reasonable expectation of success.

In regards to the newly added claims, the Applicants submit that claim 19 may be differentiated from the prior art since Kreuter, et al., alone or in combination with the other references, fail to teach or disclose that stable avidin-biotin complex is formed by incubation at 10°C. Regarding claim 20, Kreuter, et al., alone or in combination with the other references, fail to teach or disclose purification by centrifugation/redispersion. Regarding claim 21, Kreuter, et al., alone or in combination with the other references, fail to teach or disclose that the avidin which is covalently bound to the nanoparticles has 2.4 binding sites that are functionally available for forming an avidin-biotin complex.

Clearly, the combined teachings of the prior art fail to render the presently claimed invention obvious. It is therefore respectfully submitted that the present invention defined in the presently amended claims is patentably distinguishable over the prior art teachings under 35 U.S.C. 103(a). Based on the aforementioned differences, each and every element of the present invention recited in the present claims is not set forth in the cited prior art, nor would one skilled in the art be motivated to modify or combine the teachings of the prior art to arrive at the presently claimed invention. Therefore, the Applicants respectfully request that this rejection be withdrawn.

Conclusion

For the foregoing reasons, it is believed that the present application, as amended, is in condition for allowance, and such action is earnestly solicited. Based on the foregoing arguments, amendments to the claims and deficiencies of the prior art references, the Applicant strongly urges that the obviousness-type rejection be withdrawn. The Examiner is invited to call the undersigned if there are any remaining issues to be discussed which could expedite the prosecution of the present application.

Respectfully submitted,

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Original Paper

Biotin Status and Its Correlation with Other Biochemical Parameters in the Elderly People of Japan

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ABSTRACT

Objective: Biotin plays important roles in carbohydrate and lipid metabolism as well as in the decarboxylation of amino acids. In this study, to determine the biotin status in elderly people, we determined the levels of biotin and other biochemical variables in their serum.

Methods: Blood was collected from 685 elderly people aged 65 years and over (284 men and 401 women) and from 2,004 reference people. Biotin levels in the serum were microbiologically quantified by the agar plate method and other biochemical variables were recorded using the autoanalyzer.

Results: The serum biotin level in elderly people was 10.2 ± 7.20 pmol/ml (2.5 ± 1.76 ng/ml), the distribution of which was skewed to the right compared to the reference group (9.4 ± 1.43 pmol/ml) (2.3 ± 0.35 ng/ml). However, serum biotin levels did not change with age in the elderly people and no sex-related differences were detected. On the basis of the correlation coefficients among the biochemical variables in the serum, biotin levels were correlated positively with the total cholesterol level. A negative correlation was found between the biotin level and the serum albumin, triiodothyronine, phosphate, and calcium levels. On the other hand, 5.8% of the elderly people had